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A CASE OF GENERALIZED INFECTION WITH A DIPHTHEROID ORGANISM.*

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On account of our limited knowledge of diphtheria-like organisms and their effects and also because of the generally accepted custom of diagnosing diphtheria by the morphology and staining characteristics of its bacillus, the report of a case of non-diphtheric infection by an organism resembling the Klebs-Loeffler bacillus, morphologically and otherwise, must be of interest to bacteriologists. While the classical description of the Klebs-Loeffler bacillus gives us a clear and well-defined picture of a slender, curved, bipolar staining rod, tending to grow in irregular groups and often in parallel arrangement, we know that practically there is no organism which presents so varied a morphology. The short, rather thick, uniformly staining rod often seen at the beginning of the disease and also sometimes near its termination, the irregular, clubbed, involution forms, and the typical slender, bipolar forms give us a range of morphology which may well puzzle any but the most skilled and experienced diphtheriologists. And yet it is well known that, with all the bizarre forms met, there is something characteristic and convincing about the picture of a well-stained spread of Klebs-Loeffler bacilli. The various classes of pseudodiphtheria and diphtheroid organisms, however, which are believed by some to be merely atypical or attenuated Klebs-Loeffler bacilli and by others to be new and distinct types of organism, still further complicate the subject and give a varied and constantly renewed interest to the study of the bacteriology and pathology of diphtheria. It is for these reasons that I venture to present the report of a case of local and general infection with an organism so closely resembling the Klebs-Loeffler bacillus that it was so diagnosed by a number of experienced diptheriologists and yet differed from it widely in many of its cultural characteristics.

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The case which I wish to report is that of a girl, 16 years old, a housemaid who entered the Female Hospital of St. Louis, Missouri, with a diagnosis of lues. Her physical examination revealed a white girl of average development and good nutrition, with no deformities. The examination of the respiratory, circulatory, digestive, nervous, and urinary systems revealed nothing abnormal. The genital system showed no venereal sores and no signs of lues, but a slight leucorrhea was present, with no gonorrheal organisms in the discharge. On September 19, the patient suddenly developed a temperature of 104°, accompanied by nausea and vomiting, with enlargement and inflammation of the tonsils. The tonsils continued to enlarge until they practically occluded the fauces and almost prevented respiration. The diagnosis of phlegmonous tonsilitis was made and both tonsils were incised freely. The disease then ran a septic course with delirium, exacerbations and remissions of temperature, etc. Six thousand units of anti-diphtheric serum were administered September 20, but had no immediately favorable influence on the course of the disease. September 24, she developed a severe leucorrhea and three days later complained of pain about the vulva. On examination, an exudate was seen on the vulva giving the appearance of a membrane and the diagnosis of membranous vulvitis was made. The membrane soon disappeared, however, and the mucous surface showed only reddened, eroded ulcers, which bled readily on manipulation. The discharge from the vagina had at this time a very offensive odor. Patient also appeared deaf. Six thousand units of anti-diphtheric serum were again given, but again with no apparent influence on the course of the disease. On October 4, there was a profuse discharge from the right ear, having the same peculiar and offensive odor as that from the vagina. Later, both ears discharged profusely. On November 1 the vaginal lesion was apparently well, the throat completely healed, the hearing restored, and the patient feeling well, the only evidence of the condition being a slight discharge from both ears. For this clinical history, I am indebted to the kindness and courtesy of Dr. Brooks, assistant resident physician at the Female Hospital.

Bacteriology.—On September 19 a swab from the throat of the patient was spread on Loeffler's serum and sent to the City Bacteriologist for diagnosis. Only cocci and a few short bacilli were found and the report was therefore returned that *no* diphtheria bacilli had been found. On October 8 a culture was taken from the vaginal discharge and short slender bacilli were found in almost pure culture, so much resembling the Klebs-Loeffler organism both in morphology and arrangement and in their staining characteristics that a positive report of diphtheria was considered justifiable and such a report was sent with a request for subsequent cultures. Because of the curious and unusual course of the disease, numerous cultures were sent from time to time, and I began a systematic study of the organism, which was later found to be present not only in the vaginal discharge and in the vulvar exudate, but also in the discharge from the ear and throat, and in the main to the almost complete exclusion of other organisms.

The organism is a bacillus, somewhat shorter and of more regular form than the typical diphtheria bacillus, but very closely resembling it in morphology and staining characteristics. There is no tendency to the formation of threads, but the grouping is very similar to that of the Klebs-Loeffler organism. It stains readily with anilin dyes and reacts to Loeffler's methylene blue very much as does the diphtheria bacillus. With both Loeffler's methylene blue and with the Neisser stain, some of the organisms show distinct polar bodies, but most are either unipolar or stain uniformly throughout. With the Gram stain, the stain washes out, but with considerable difficulty, so that in every preparation, many of the organisms retain the stain; for this reason it may well be regarded as gram doubtful. The organism is actively motile, especially in young broth cultures, and in this respect differs markedly both from the Klebs-Loeffler bacillus and from most of the diphtheroid organisms. It grows readily on most of the ordinary media, growing slowly at room temperature, while the optimum temperature is 37°. On Loeffler's serum, which seems the most favorable media for its cultivation, it forms a moist, soft, slimy, abundant, spreading growth, which is at first nearly colorless, but later becomes pale yellow and in a few days completely liquefies the serum. On potato, it forms a moist, elevated, rapidly spreading and abundant, almost colorless growth. On slant agar, it forms a slightly yellowish, moist, spreading growth with a tendency to soften and liquefy the agar. On blood agar, a small area of hemolysis surrounds a fairly large, elevated, yellowish, moist colony. On glucose agar, a very small amount of gas forms. In glucose broth, there is abundant gas formation, but none in lactose, maltose, or saccharose broth. All these sugar media gave an alkaline reaction after 72 hours. On plain broth there is a rapid growth, rendering the fluid at first cloudy, but later most of the growth settles to the bottom and sides as a granular, grayish-white precipitate. The gelatin stick culture rapidly liquefies the gelatin; an inverted cone is formed at first, but in 48 hours the entire tube is liquefied and most of the growth has settled to the bottom. In litmus milk there is slow acid formation and the milk is coagulated at the end of 72 hours. In all of the media, there is a characteristic, peculiar, sweetish, acetone-like odor, which is said to be similar to that noted in the patient. The 48-hour culture on peptone gives a pronounced indol reaction and a slight phenol reaction, but none for nitrites.

A guinea-pig injected intraperitoneally with 1 c.c. of a 48-hour broth culture showed no symptoms, but on repeating the injection a week later on the same pig, using 3.5 c.c. of a 24-hour culture, the pig died within about eight hours and the abdomen was distended with a slightly cloudy fluid in which the organisms were very abundant. Subcutaneous inoculations with both 24- and 48-hour cultures caused an induration at the point of inoculation; this increased in size and symptoms of illness, fever, weakness, etc., developed. Finally the indurated area softened and ruptured. The first pig rapidly recovered after the rupture of the abscess, therefore the second pig was killed soon after the breaking of the abscess and cultures were taken from the pus of the abscess, the peritoneal fluid, the heart blood, and the throat. No growth developed in the culture from the heart blood, but all the other cultures contained the characteristic organism and in nearly pure culture.

Forty-eight-hour broth cultures were filtered through a Reichel filter and the sterile filtrate inoculated intra-abdominally into guinea-pigs. These died in 36 to 48 hours and no organisms were recovered from the bodies. Hence the organism produces a toxin which is capable of killing guinea-pigs.

This organism differs from the Klebs-Loeffler bacillus then in the following particulars: (1) It is motile, while the diphtheria bacillus is non-motile. (2) It is somewhat less irregular in shape and staining than the typical Klebs-Loeffler bacillus and tends to grow shorter with age and to lose its polar staining, while the diphtheria bacillus tends to grow longer and to develop larger and more distinct polar bodies and also bizarre involution forms. (3) Both organisms grow best on serum and at 37°C., but the diphtheria colonies are dry and granular, while the colonies in my case are moist and spreading and eventually liquefy the serum. (4) On agar, the differences are similar to those on serum. (5) Gelatin is never liquefied by the Klebs-Loeffler bacillus but is rapidly liquefied by the organism in my case. (6) The diphtheria bacillus tends to produce acid, while the organism I am describing produces an alkaline reaction in most media. (7) Diphtheria bacilli produce no gas in glucose broth, while mine produce abundance of gas in glucose broth, but none in lactose, maltose, or saccharose broth. (8) Milk is slowly coagulated by the organism in my case, while it is unchanged by the Klebs-Loeffler bacillus. (9) The diphtheria bacillus produces no indol, while mine produces an abundance of indol.

The organism I am describing was very pathogenic for the patient, but produced only a local abscess, with very slight general symptoms when injected subcutaneously into the guinea-pig; when injected intraperitoneally, it had no effect unless a considerable amount was used. As to contagion, while the patient was evidently able to reinfect herself, there is no history of others acquiring the infection from her. Her blood serum agglutinated the organism in dilutions of one to 50 and more slowly and less completely in dilutions of one to 100. A comparison of the morphological and cultural characteristics of this and a number of related organisms is given in the table which follows.

Proteus vulgaris has been inserted in the table because the odor noted in the patient and in most of the cultures, the liquefying action on serum, and the motility remind one somewhat of the proteus group. The morphology and arrangement, however, and most of the growth characteristics, as may be seen in the table, are quite different and place this organism indubitably in one of the many groups of diphtheroid organisms. Of all the diphtheria-like organisms which have been described, the one which my organism most resembles is the one called "*B. diphtheroides liquefaciens*" by Graham-Smith,[1] this being motile and liquefying serum. A similar

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[1] *The Bacteriology of Diphtheria*, edited by G. H. F. Nuttall and Graham Smith, Cambridge, 1908.

	Organism in M. H.	Klebs-Loeffler	Hoffman	Xerosis	Graham-Smith B. Diph. Liquefaciens	Klein's B. Diphtheroid	Hamilton's Chromog. Bacillus	Ruediger Diph. Bacillus	Proteus Vulgaris	Colon Bacillus
Shape........	Short and slender	Long ,slender, curved, irregular	Short, straight, oval. Median light band	Diphtheria-like, long, curved, and larger at ends	Long, slender, slightly curved	Diphtheria-like	Short, solid, granular rods	Regular, short, and long; granular	Short and plump or long and slender	Short, plump rod
Size..........	....	1.5–3.5μ long 0.4–1.0μ diameter	1–1.5μ long .12–.2μ diameter	....	....	....	....	....	1–1.2–4μ	1–3μ long $\frac{1}{3}$–1μ thick
Stain (anilin dyes)......	Deep and ready	Deep and ready	Deep, uniform, except light band	Ready	Ready	Diphtheria-like	....	....	Often irregular with light spot in center	Ready stain
Neisser stain..	Often +	Bipolar +	Occasional	Positive	Positive	Positive	1. Negative 2. Positive	Neisser positive	....	....
Gram stain...	Doubtful	Positive	....	Positive	Positive	Positive	....	....	Negative	Negative
Motility......	Actively motile	Non-motile	Non-motile	Non-motile	Motile	Segments	....	....	Actively motile	Motile
Grouping.....	No chains, irregular grouping	Irregular grouping	Irregular grouping	Irregular	Irregular	Irregular	Irregular	....	Chains	....
Involution forms......	Common and varied	Common and varied	May or may not be present	May be present	May be present	Present	....	....	Common	....
Serum........	Moist, grayish yellow. Liquefying	Grayish white, dry, and granular	Small, round, elevated, and pure white	Scaly, adherent colonies. Slow growth	Yellowish colonies; slow liquefaction	Granular colonies; liquefies	Abundant growth; liquefaction	Abundant growth	Often liquefied	....
Agar streak...	Grayish white, moist, spreading	....	....	....	Abundant, moist, yellowish growth	Slow growth	Greenish growth. 1. Agar purple 2. Purple growth	Abundant, many, moist, spreading	....	Uniform layer
Agar colonies.	Elevated, yellowish, moist, distinct	Large, translucent, with raised centers	Large, white, elevated, shining, moist, round	Small, gray, adherent	....	....	....	....	Stellate colonies	Grayish colonies

Gelatin.......	Cone-shaped depression, later complete liquefaction	No liquefaction	No liquefaction	Little growth; no liquefaction	Liquefaction	Growth	....	Abundant	Liquefaction	Rapid growth, no liquefaction
Broth.......	Diffuse clouding	Whitish, granular pellicle. Snowstorm through fluid	Diffuse, cloudy, settling on sides	Clear, granular deposit	Cloudy, with granular deposit	Very little growth	Cloudy	Cloudy, alkaline	Rapid, cloudy pellicle	Greenish, cloudy, rapid growth
Potato.......	Moist, colorless	Slight, dry, invisible	Copious, but invisible	Invisible growth	Abundant, white growth becoming yellow	No growth	1. Brown growth 2. Purple growth	Hardy, light brown growth	Dirty, yellowish growth	Abundant
Litmus milk..	Slightly acid coagulation in 72 hours	Acid, but no coagulation	No acid and no coagulation	No acid and no coagulation	Coagulation	Coagulation	....	Turns white in 5–6 days	Initial acid and coagulum. Later proteolysis	Acid coagulation
Glucose broth.	Gas (much)	No gas	No gas	No gas	No gas	....	Acid	Acid	Gas	Gas
Lactose broth.	No gas	No gas	No gas	No gas	No gas	....	....	....	No gas	Gas
Maltose broth.	No gas	No gas	No gas	No gas	No gas	....	....	....	No gas	Gas
Saccharose broth......	No gas	No gas	No gas	No gas	No gas	....	....	....	Gas	No gas
Optimum temperature....	35°–37° C.	35°–37° C.	36°–37° C.	....	....	....	....	....	25°	37.5° C.
Indol reaction.	Positive	Negative	None	....	....	....	....	....	Positive	Positive
Reaction of sugar media	Alkaline	Acid	Alkaline	Acid	Alkaline	....	Acid	Acid	Alkaline	Acid
Odor of cultures.......	Sweet, acetone-like odor	None characteristic	....	....	....	....	....	....	Putrefactive odor	Fetid odor
Virulence.....	Path. to patient; subcutaneous abscess in G.-pig. Noeff. intraperitoneally except in large doses	Pathogenic	Non-pathogenic	Non-pathogenic	Non-pathogenic	Produces subcutaneous abscesses	1. Non-pathogenic 2. Produces septicemia	Very virulent	Not virulent	Varies greatly

organism has been described by Hamilton, although it is difficult to say whether it is identical. The Graham-Smith bacillus, however, forms no gas in glucose broth and is non-pathogenic. In none of the diphtheroid organisms described have I found mention of a special odor, which is so characteristic and distinctive a feature of my organism. Hamilton, in a thorough study of pseudodiphtheria organisms found in cases of otitis media, attempts a classification of these organisms into the following three classes, basing her classification on their fermenting power. Her first class ferments dextrose and saccharose, but not maltose, lactose, or dextrin. It consists of short, uniformly staining rods, which are usually non-virulent. The second class ferments dextrose and maltose, but never saccharose. The organisms of this class closely resemble Klebs-Loeffler bacilli and are often virulent. The third class is typified by the Ruediger bacillus, which is a non-fermenting bacillus. The last-named bacillus she found but three times in an examination of 142 cases of otitis media, and the other two classes were represented in 51 cases. She concludes that these organisms are frequently the cause of otitis media. The fermenting power of these organisms makes a convenient basis of classification, although even on this basis it is difficult to include all of the diphtheroid organisms under any one of these three classes. My organism might be placed under her class 2, but for the fact that it does not ferment maltose. As to pathogenicity, the diphtheroid organism in my case, which was found practically in pure culture, appears to have been the cause not only of an otitis media, but also of tonsilitis and peritonsillar abscess and of an exudative vaginitis and vulvitis, together with fever, delirium, and other indications of a general intoxication.